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Responses of Zooplankton and 'Chaoborus' to Temephos in a Natural Pond and in the Laboratory

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16. ABSTRACT

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Responses of Zooplankton and *Chaoborus* to Temephos in a Natural Pond and in the Laboratory

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Abstract. Application of the organophosphorus insecticide temephos to a natural pond in central Minnesota was followed by reduction within 24 hr in all cladocerans, in *Diaptomus leptopus* and in *Chaoborus americanus*, and increases in cyclopoid copepods, copepod nauplii and the rotifer *Keratella cochlearis*. *Daphnia pulex* that reappeared 35 days post-application were ex-ephippial. After application, reproduction of cladocerans and *Diaptomus* was markedly reduced compared to the previous year, an effect attributed to temephos. *Daphnia* population density was strongly reduced into the fall season, long after the spring applications, compared with both a reference pond's and the previous season's populations. On-site bioassays demonstrated 24-hr mortalities of *Daphnia* and *Chaoborus* that were comparable to the population decreases in the pond. Laboratory toxicity tests showed *Daphnia* was the most sensitive followed by *Chaoborus* and *Diaptomus*. Comparable results were seen in the pond population changes, *in situ* bioassays and laboratory toxicity data for *Daphnia* and for *Chaoborus*, but sensitivities in the pond were somewhat greater than in the laboratory. Risk assessment research calls for long-term field reproduction analysis to avoid a false "recovery" report, combined with laboratory life cycle analysis and short acute exposure tests.

This report summarizes a two year field study on the effects of temephos on zooplankton and the phantom midge larva *Chaoborus americanus* in natural ponds in central Minnesota. The purpose was to compare laboratory and on-site bioassays with responses of field populations, and to provide a full season's data on non-target populations, following exposure to concentrations of temephos used for mosquito control.

The organophosphorus insecticide temephos (ABATE®), or *0,0,0′,0′*-tetramethyl-*0,0′*-thiodi-*p*-phenylene phosphorothioate, is used as a larvicide for control of blackflies and mosquitoes, and less frequently for "nuisance" midge larvae. Suggested application rates of 50–500 g/ha active ingredient are designed to distribute 0.5 to 5 μg/L onto wetlands or streams. For various species of mosquitoes, 24-hr LC_{50}'s of 1.03–3.0 μg/L (Helson *et al.* 1979) and 0.7–1.7 and 1.0–2.1 μg/L (Rettich 1979) have been reported. For non-resistant chironomid midges, 24-hr LC_{50}'s ranged from 16–21 μg/L, for resistant midges, from 29–7200 μg/L (Ali and Mulla 1980).

With the exception of disease vector copepods, little laboratory or field work has been reported on the effects of temephos on zooplankton, and no long-term field studies with repeated applications. *Cyclops vernalis*, the cyclopoid that transmits the parasitic nematode guinea worm *Dracunculus medinensis* to humans, had a 24-hr LC_{50} of 2 μg/L (Muller 1970), while *Cyclops spartinus* had no mortality at even 1,000 μg/L (Ruber and Baskar 1968).

Responses of natural zooplankton populations to applications of temephos in field tests have been reported, and cladocerans appear more sensitive than copepods. Application of temephos granules for the control of chironomids resulted in a 50% and 85% reduction of *Daphnia pulex* and *Daphnia galeata*, respectively, within one week followed by a

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three month absence, while the copepod *Diaptomus sp.* was not affected (Ali and Mulla 1978). Papst and Boyer (1980) reported a decline in *Daphnia sp.* and *Diaptomus sp.* following temephos treatment, while rotifers increased strongly, an effect they attributed to the secondary increase in algae following depletion of the larger herbivorous zooplankters. Rettich (1979) found 100% mortality of *Cyclops sp.* at concentrations >50 μg/L and *Daphnia sp.* at >100 μg/L. Treatment of small artificial ponds with temephos ranging from 7.5 to 1.8 μg/L over 24 hr reduced cladocerans to near zero within 24 hr, with no recovery in 40 days, with copepods absent for 20 days (Hughes *et al.* 1980). Frank and Sjogren (1978) reported no evidence for any effect of temephos on the occurrence of copepods, cladocerans, or other crustaceans.

The phantom midge larva *Chaoborus* can play a central role in the pond ecosystem, yet no toxicity data for temephos and *Chaoborus* are available. These larvae prey actively on zooplankton and other small invertebrates, including first instar mosquito larvae, and are extremely important in structuring zooplankton communities, because of their size-selective predation (Vinyard and Menger 1980; Lynch 1979; Zaret 1980). They may act to exclude mosquito larvae (Sailer and Lienk 1954), and show a high strike efficiency on the early instars (Helgen, in prep).

This report presents population data for cladocerans, copepods, rotifers and *Chaoborus americanus* gathered during the ice-free season of the year preceding the two spring temephos treatments (1983) and into the October following treatment. A reference pond was sampled during the treatment year, 1984. On-site bioassays, conducted at the time of each treatment, and laboratory toxicity tests produced data for some species of zooplankton and *Chaoborus*. Data on zooplankton reproduction indicate a potential for depletion of future populations, a prediction that would not be made if densities of adults alone were used to indicate recovery from the insecticide.

This work is part of a larger study undertaken during 1983 and 1984 by the U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota, to examine the ecological risk assessment procedures used in the pesticide registration process.

Methods

The study ponds are located in the Crow-Hassan Park Reserve in northwestern Hennepin County, Minnesota. Both are mud-bottomed seepage ponds, the treated pond with an area of 0.4 ha, with average depth 90 cm, and maximum depth 130 cm, and the nearby reference pond with 0.56 ha area, and 80 cm average and 92 cm maximum depths. The treated pond has populations of common pond invertebrates and fish are absent. Its shoreline vegetation is dominated by *Typha latifolia* (cattails) and *Phalaris arundinacea* (reed canary grass). The latter two plus *Sagittaria* (arrowhead) dominated the reference pond, which had greater midsummer densities of *Lemna* spp. (duckweed). There was no history of pesticide applications, and the ponds do not receive agricultural runoff. Two wooden walkways extending into the pond from opposite sides allowed sampling without disturbance of the ponds' sediments.

Temperature, dissolved oxygen, conductivity and pH were monitored continuously at a depth of 30 cm during 1984 in the reference pond and for both study seasons in the treatment pond with a battery-powered Hydrolab Water Quality Measurement System (Hydrolab Corp, Austin, Texas). Dissolved oxygen determinations were made by the modified Winkler method from 12 July through September, 1983. The first temephos application (22 May), made with an emulsified concentrate formulation, was delivered by backpack sprayer at a rate of 0.5 g/ha active ingredient. On 5 June, a sand granule formulation was applied at the same rate by helicopter. Water samples for analysis of temephos were removed from locations off both walkways before treatment and at 1, 2, 4, 8, 12, 24, 48, 72 hr following the first application and at 2, 4, 8, 12, 24, and 48 hr after the second application. All samples were analysed with a Hewlett-Packard model 1084B high pressure liquid chromatograph (Siefert 1985). The limit of detection for temephos was 0.7 μg/L.

Zooplankton Collection and Analysis

Zooplankton populations were surveyed during the 1983 ice-free season, and in 1984 from 5 May to October. Zooplankton were collected by two different methods. First, triplicate horizontal plankton tows were made at 1100–1300 hr with an 80 μm mesh, 20 cm diameter Wisconsin-style plankton net towed approximately 50 cm below the surface, 46.8 m across the treatment and 32.2 m across the reference ponds. Secondly, six funnel trap samplers were placed on the bottom of each pond, three off each walkway, between 1700 and 2000 hr CDT and collected the following day between 0700–0900 hr. Horizontal plankton tows were taken weekly from 5 May through 1 October, 1984. A more intensive sampling schedule surrounded each application date, with plankton tows taken one day prior to application, just before and 8 hr after the application on the treatment day, and 1, 3, 5, and 7 days following application. In 1983, horizontal plankton tows were taken from 25 May to 29 June. Use of the funnel trap samplers, three off one walkway, was initiated on 11 July 1983 after heavy growth of macrophytes and *Lemna* interfered with plankton tows, and continued through 7 October 1983. Plankton tows were resumed on 15 September after macrophyte density had lessened, and continued through 13 October, 1983. In 1984, plankton tows were taken throughout the summer. Funnel trap plankton samples were taken from 5 May through 18 September during 1984.

The Wisconsin net was suspended horizontally under an aluminum frame supported by two floats and connected to a rope mounted on pulleys on the two opposing walkways. Assuming unimpeded flow through the nets, the volume of water filtered per tow in the treatment pond was 2.0 m^3, in the reference pond 1.34 m^3. The funnel trap samplers (modified from Whiteside and

Lindegaard 1980), consisted of four funnels mounted in a 30 cm plexiglass square so that the stems project through into 8 oz collecting jars. Masonite strips mounted at the level of the funnel openings prevented the traps from sinking into the mud. Zooplankton migrate upwards at night into the jars which were filled with 80 μm mesh filtered pond water before placement. The contents of the four jars per trap were pooled, concentrated, and preserved in 4% sucrose-formalin. The area covered by the four funnels was 0.0283 m^2 per sampler.

Zooplankton counts were made either with five one ml subsamples counted in a one ml Sedgewick-Rafter slide at 63× or with 4 to 5 subsamples of 1, 2, or 5 ml counted in a plankton wheel at 34×. Samples were counted with subsample volumes and sample dilutions chosen so that at least 400 individuals or 25 per cent of the volume was counted. Although identifications were usually to species, some groups are pooled for the results reported here. Egg counts were made for the cladocerans and copepods, and the number of egg-bearing females recorded. Fewer reference pond samples were counted.

Bioassays in situ

In situ bioassays were carried out on both treatment dates. Exposure chambers were prepared and placed in the pond one day before application. Mortalities were recorded at 4, 24, and 48 hr post-application. If all animals were dead at the time of observation, another exposure was started. The exposure chambers, holding approximately 300 ml pond water when floated in place with a styrofoam collar, consisted of a cylinder of stainless wire screen (15 wires/cm) mounted into the glass base and screened, capped top of a cut glass jar.

In situ bioassays were carried out along each of the two walkways in the pond. Animals for the *in situ* assays were collected prior to the first treatment (22 May) with a 151 μm mesh plankton net from the site of the assay. After the treatment, test animals were collected from nearby ponds. For each application date three taxa, *Daphnia pulex*, *Diaptomus sp.* and *Chaoborus sp.*, were tested.

Laboratory Toxicity Tests

Laboratory toxicity tests were performed from Dec 1983 to Dec 1984 with animals from the study pond and from local ponds near the Environmental Research Lab in Duluth, MN. The tests, conducted at 20–23° C, consisted of two types of exposures: a static test of 24–96 hr duration, and a short time exposure test. In the static test, animals were exposed for a set time, usually 24, 48, or 96 hr. Test animals were sorted randomly into groups of 10 in beakers containing Lake Superior water. To start the test, the water was replaced with a series of five aqueous dilutions of temephos prepared by diluting a 0.5% methanol stock. Controls contained no methanol. Temephos was analyzed at the end of each test. Each test included duplicates and Lake Superior water controls containing no temephos. Animals were observed for mortality at frequent intervals during the first 12 hr, and daily thereafter.

In the short-term test procedure, *Daphnia pulex* was exposed for 4, 8, and 12 hr and transferred to temephos-free water for observation. Ten *Daphnia* per test were randomly distributed into vials fitted with stainless mesh screen windows. At time zero, vials were transferred to a 300-ml exposure chamber containing temephos. Exposure was terminated by draining and rinsing the vials four times in fresh water. The *Daphnia* were then transferred to 80 ml of control water and mortality was recorded at 24 hr.

Results

Zooplankton

In the treated pond, high mortalities of the cladocerans *Daphnia pulex*, *Simocephalus sp.* and Chydoridae, and of the calanoid copepod *Diaptomus leptopus* were seen within 24 hr of the first treatment (Table 1). In contrast, there were marked increases in cyclopoid copepods, copepod nauplii, and the rotifer *Keratella cochlearis*. No cladocerans were found by the time of the second application, 14 days after the first. Again, *Diaptomus* and *Chaoborus* experienced mortality, while the cyclopoids, copepod nauplii and *Keratella* showed increases 24 hr after the second application.

Daphnia pulex showed high sensitivity to temephos, with 97% mortality in the pond population within 8 hr of the first application (Table 1). *Daphnia* reappeared in the plankton samples 35 days after the first treatment (Figure 1A) as juveniles, most likely ex-ephippial since no adults were present. *Daphnia* densities at the end of June 1983, the year prior to treatment, were at least 10 times greater than in late June 1984, one month after treatment, and three times greater in June 1984 in the reference pond than in the treatment pond. Ephippial females were noted in the 11 and 21 May 1984 samples (Figure 1C), and free floating ephippia were seen in the pond just before the first treatment. Egg production was not seen again until mid to late July, with a few ephippia-bearing females noted on 10 July (Figure 1C). The large increase of *Daphnia* in the reference pond from the end of July into August 1984 did not occur in the treated pond. By October, the treated pond had no *Daphnia* when the previous fall there was an increasing population. The reference pond in the fall had a strong *Daphnia* population.

Compared with 1983, 1984 midsummer reproduction by *Daphnia* was depressed (Figure 1B). In 1983, the number of gravid females/m^3 increased to 1540 before plankton tow sampling ended at the end of June; in 1984 the maximum seen after treatment was 105 in July. The maximum number of gravid females in the reference pond was 977/m^3. In the fall of 1984, there were no gravid *Daphnia* in the treated pond, and adult densities were low compared to the fall of 1983 when some reproduction occurred. In the reference pond there were gravid

Table 1. Pond densities of zooplankton and *Chaoborus* just before and up to 96 hr following 2 treatments with temphos. Treatment I, 22 May and Treatment II, 5 June 1984. Densities are per m^2 for funnel trap (FT) data and per m^3 for plankton tow (PT) data, with standard deviations[c,d]

Taxa	Sampling method[b]	Pre-Trt		8-hr Post-Trt		24-hr Post-Trt		72-hr Post-Trt		96-hr Post-Trt	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Treatment I											
Daphnia pulex	PT	2630	717	37[a]	40	82[a]	143	0	0		
Chydoridae	FT	3600	3580	—	—	2370	872	0	0		
Simocephalus	FT	2300	985	—	—	0	0	0	0		
Diaptomus leptopus	PT	1160	627	167	268	268[a]	263	130[a]	66		
Cyclopoida	FT	34200	27800	—	—	68000	30600	25400	14300		
Copepod nauplii	FT	20300	10800	—	—	36000	20400	20400	6740		
Keratella	PT	64	21	78	20	120	70	163	61		
Chaoborus	PT	354	252	56	79	56	20	20	11		
Treatment II											
Daphnia pulex	PT	0	0	0	0	0	0	0	0	—	—
Chydoridae	FT	0	0	—	—	0	0	—	—	188[a]	.306
Simocephalus	FT	0	0	—	—	0	0	—	—	0	0
Diaptomus leptopus	PT	937	353	320[a]	61	762	233	1358	63	—	—
Cyclopoida	FT	91500	91700	—	—	142000	167000	—	—	113000	76500
Copepod nauplii	FT	33700	32500	—	—	57400	44700	—	—	95600	81300
Keratella	PT	1460	774	78[a]	20	3387[a]	786	3420	1080	—	—
Chaoborus	PT	138	44	25[a]	25	15[a]	13	17[a]	29	—	—

[a] Indicates significant ($p \leq .05$) difference from Pre-Dose density as determined by paired t-test with 4 d f for plankton tow data, 8 d f for funnel trap data

[b] PT = horizontal plankton tow, FT = funnel trap

[c] 0 means none present

[d] means no sample taken

Daphnia in the fall (33, 49/m^3). The decrease in reproduction appears to result from an effect on the number of gravid females rather than on brood size (Figures 1B, C).

Simocephalus sp. declined to zero within 24 hr of the first treatment, and was not present at the second treatment (Table 1, Figure 2A). The cladoceran reappeared 35 days later and increased in August, but the density was less than 17% of the population in 1983. Almost none of the *Simocephalus* appearing in July and August of 1984 were gravid (Figure 2C). Some gravid females were collected in the plankton tows at the end of August, but few were found in the funnel trap samples. The fall increase in gravid females seen in both the reference pond and in 1983 before treatment did not occur in 1984 in the treated pond. As with *Daphnia*, when reproduction occurred, brood size was comparable to that of 1983.

Cladoceran populations within the family Chydoridae had a reduction of 34% with 24 hr after the first treatment, and were not found at the second treatment (Table 1). The production observed in July, 1983 was not apparent in 1984. The reference pond data is insufficient for comparison. Although the numbers of Chydoridae seemed to recover from treatment (Figure 3A), reproduction declined (Figure 3B). After 25 May, no gravid Chydoridae were collected with funnel traps through September. The number of gravid females collected with plankton tows after July 25 was about one-half that of the 1983 population, except on 9 Sept., when the number of gravid females was comparable to 1983.

Populations of *Chaoborus americanus* had decreases of 84% and 89% within 24 hr of the first and second treatments. Compared with 1983, *Chaoborus* had reduced numbers in 1984, until the end of June (Figure 4). By 19 June, 28 days after the first treatment, *Chaoborus* density was 17% that of 1983. By the end of June, 38 days post-treatment, densities were comparable to pre-treatment and 1983 densities (Figure 4). It is not known if the *Chaoborus* collected before the second treatment were new recruits, or survivors.

Population reductions of the calanoid copepod *Diaptomus leptopus* were 77% and 19%, 24 hr following the first and second treatments. After these decreases, the June 1984 density was similar to that of June 1983 (Figure 5A), an apparent recovery.

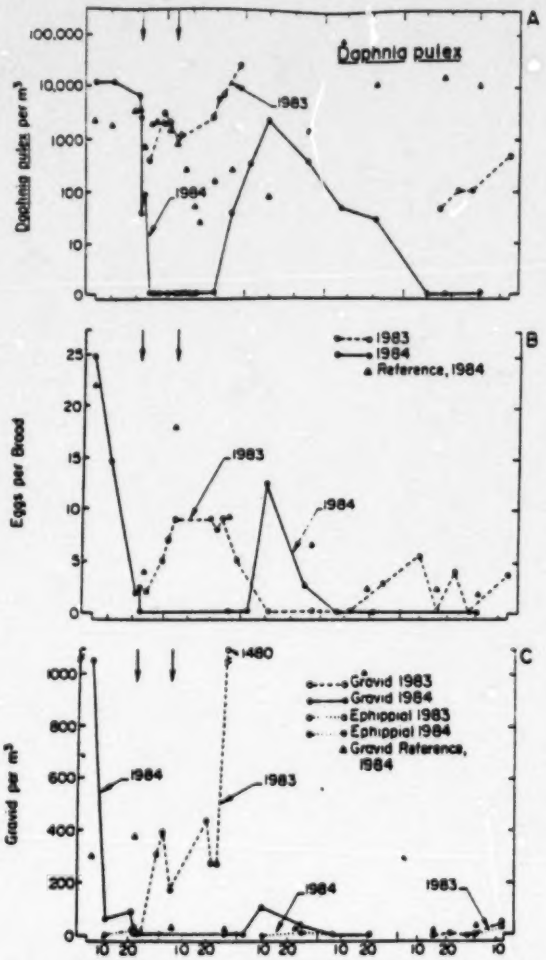

Fig. 1. Population densities and reproduction of *Daphnia pulex* in 1983 and 1984 from plankton tows in treated (○, ●) and reference (▲) ponds. A, shows total individuals per m³, B, average eggs per brood, and C, gravid and ephippial females. In B and C, zero points indicate presence of only non-reproducing individuals. No plankton tows were taken July–August, 1983, and eggs per brood data (B) for this interval are from funnel trap samples. Arrows indicate the two temephos applications

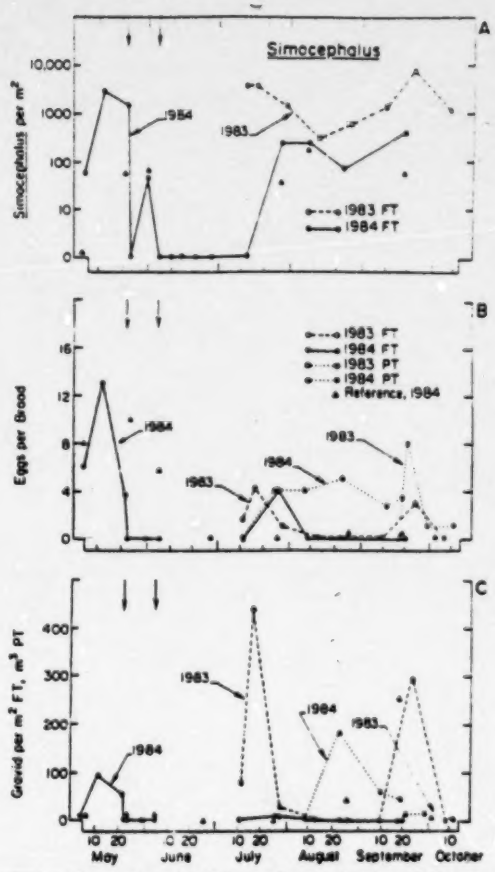

Fig. 2. Population densities and reproduction of *Simocephalus* in 1983 and 1984 from funnel trap (FT) data from treated (○, ●) and reference (▲) ponds. A, shows total individuals per m², B, average eggs per brood, and C, gravid females. Late season plankton tow (PT) data for 1983 and 1984 is included in B and C

However, although the density of *D. leptopus* was similar in June 1983 and 1984, the number of egg-bearing females was sharply depressed in 1984, with a maximum of 185/m³ compared to 900 egg-bearing females/m³ in 1983 (Figure 5B). Following the treatments, the average number of eggs per female was 28 in June, 1984 compared with 38 in June, 1983 (Figure 5C). The result is that on comparable dates in June, 1983 there were 34,200 *Diaptomus* eggs/m³ and in June 1984 just 5,200 eggs/m³, and 85% reduction in production. *Diaptomus* was not present in the reference pond.

Within 24 hr following each treatment with temephos, cyclopoid copepods, copepod nauplii, and the rotifer *Keratella cochlearis* populations approximately doubled. Both cyclopoids and total copepod nauplii (Figure 6) show a decline five days following each treatment. Funnel trap data obtained from July to Sept. 1984 show densities very similar to those in 1983, except for a possible increase in nauplii in Sept. 1984. *Keratella cochlearis* (Figure 7) increased substantially with 24 hr following both treatments. The rotifers were beginning a spring period of rapid population increase, doubling approximately every three days, as they had in 1983.

Averaged concentrations of temephos measured in five locations in the pond ranged from 4.2 to 0.9

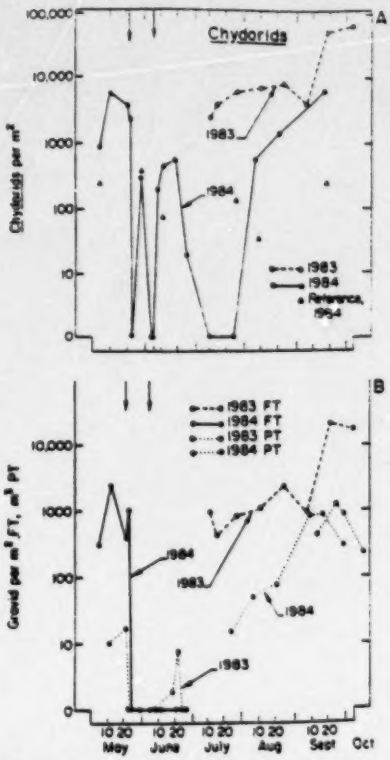

Fig. 3. Population densities of Chydoridae in 1983 and 1984 from treated (○, ●) and reference (▲) ponds. A, shows total individuals per m² from funnel trap (FT) samples. B, gravid females from FT and plankton tow (PT) samples

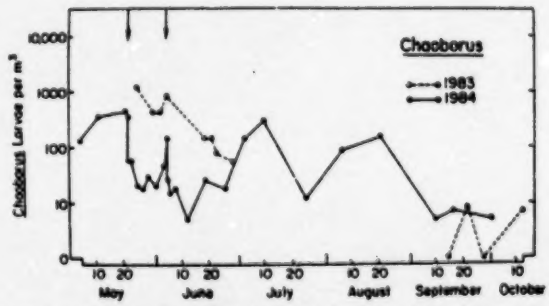

Fig. 4. Population densities of *Chaoborus americanus* larvae in 1983 and 1984 from plankton tows

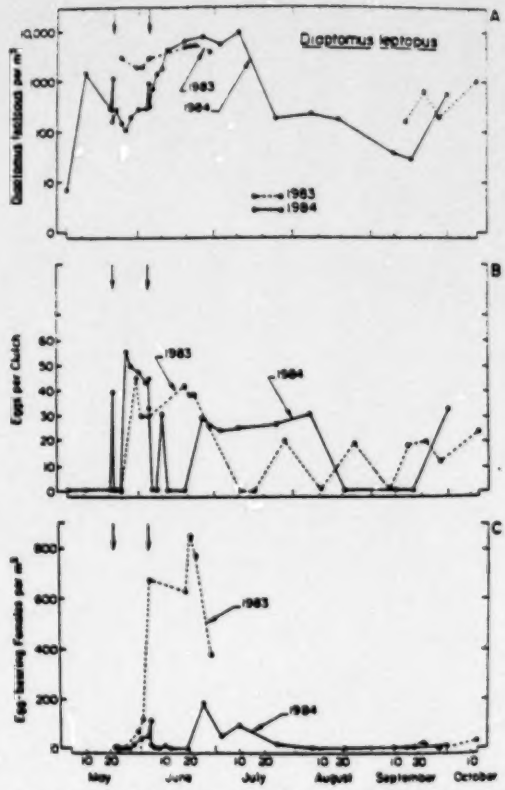

Fig. 5. Population densities of *Diaptomus leptopus* in 1983 and 1984 from plankton tows. A shows total individuals, B average eggs per clutch and C the numbers of egg-bearing females. No plankton tows were taken in July and August, 1983; eggs per clutch data (B) for this interval are from funnel trap samples

μg/L over 24 hr following the first treatment, and from 1.3 μg/L to below detection following the second. At the first hour after the first treatment, concentrations of temephos were uneven among the five sampling locations, by 8 hr the temephos was distributed evenly in the pond (Table 2). By 12 and 24 hr after the second application, temephos was found at only one site. By 48 hr after both applications, only one sample contained temephos (at 0.8 μg/L).

Average daily water temperatures were quite similar in 1984 in both the treated and reference ponds (Figure 9). Slightly warmer spring temperatures were seen in 1983 in the treated pond when averaged temperatures in May ranged from approximately 18 to 22° C compared to 13 to 21° C in 1984. Temperatures were similar in both years later in the season, although for intervals of several days in June and July of 1983, daily average temperatures exceeded the maxima seen in these months in 1984. Temperatures were higher in August during 1984.

Daily average dissolved oxygen concentrations for 1984 for both ponds are shown in Figure 9. In the treated pond, dissolved O_2 continued to increase for approximately 10 days following the first treatment. From 30 June to 6 July in 1984, dissolved O_2 in the treated pond fell below 1.0 mg/L

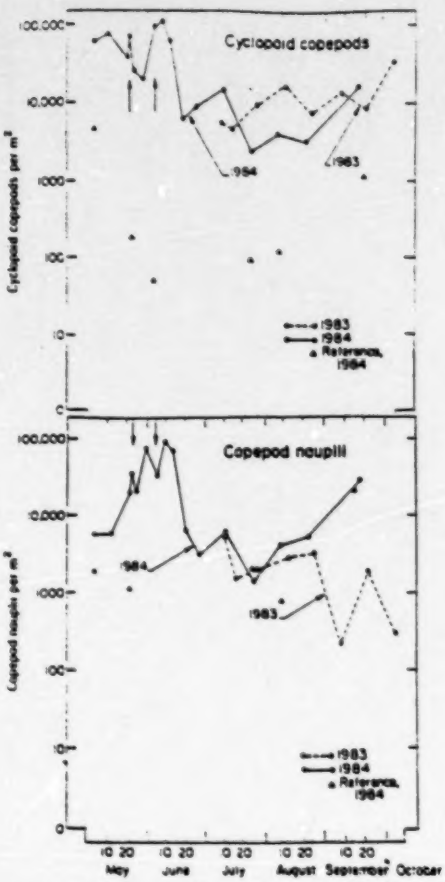

Fig. 6. Population densities of cyclopoid copepods and total copepod nauplii in 1983 and 1984 funnel trap samples

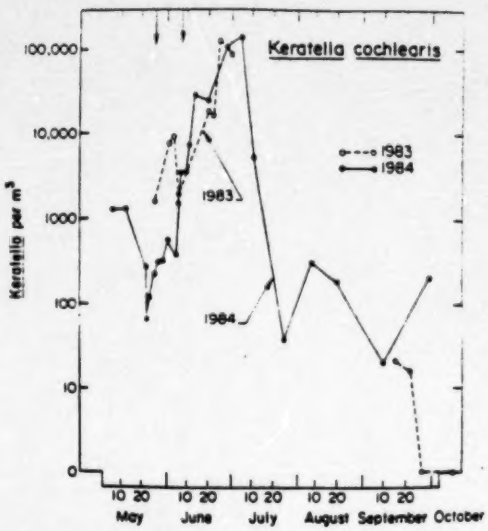

Fig. 7. Population densities of *Keratella cochlearis* in 1983 and 1984 from plankton tows

for at least five hr each day, and levels this low did not recur until 20 August to 6 September when levels fell below 1.0 mg/L for an average of 9 hr of each day. Limited data from 1983 show minimum daily O_2 below 1.0 mg/L for only a few days in the first half of July, and again at the end of July and early in August. Dissolved O_2 levels were lower in late August to early September 1984 than in 1983. In the reference pond, oxygen was substantially lower, especially during 20 to 28 May when it was less than 1 mg/L for an average of 14.7 hr per day, and 7 to 13 June, when it was at zero.

Bioassays in situ

Results of the *in situ* bioassays (Table 3) show *Daphnia pulex* the most sensitive of the animals exposed. Four hr after the first temephos application, 90% of the animals were dead or immobilized. The average concentration during the first 4 hr was 1.6 μg/L (Table 2). All *Daphnia* were dead after 18 hr exposure in bioassay chambers which were replaced at 6 hr post-application and counted at 24 hr. During this time period, the concentrations ranged from 1.9 to 0.7 μg/L, with an average concentration of 1.2 ± 0.3 μg/L (n = 15). All *Daphnia* were dead in exposures run from 24 to 48 hr post-application. At 24 hr when these bioassays were set, temephos was at 0.9 μg/L, but by 48 hr, none was detected (Table 2).

High mortality was seen after the second application. At 4 hr, 60% of the *Daphnia* were dead or immobilized. At 24 hr, all caged animals were dead and the containers were reset with unexposed animals. By 48 hr, all the daphnids were dead, the effect again at concentrations below the detection limit of 0.7 μg/L.

Chaoborus was also affected by temephos in the bioassays (Table 3). After 24 hr of exposure, 90% of the *Chaoborus* were dead. Five bioassay chambers were reset and after 19 hr of exposure to concentrations below the detection limit, only 4% of the animals were dead. After the second application, location was important. At one site by 24 hr 13% were dead, increasing to 26% by 48 hr. At another site there was 80% mortality at 24 hr, and in reset chambers 45% death in the next 24 hr (Table 3).

Higher temephos concentrations were found in the pond after the first application than after the second. Yet higher mortality (to 53% at 48 hr) was

Table 2. Temephos concentrations near the exposure containers after the first and second applications

Sampling schedule	Location # 1	2	3	4	5	X ± 1 Std Dev[a]
Predose	NF	NF	NF	NF	NF	
1 hour	17.9	0.7	1.5	0.8	NF	4.2 ± 7.7
2	1.3	2.6	2.0	—	0.9	1.7 ± 0.7
4	2.6	2.4	1.5	1.5	1.5	1.9 ± 0.5
8	1.3	1.4	1.2	1.3	1.2	1.3 ± 0.1
12	1.4	1.9	1.3	1.5	1.2	1.5 ± 0.3
24	0.8	0.9	0.9	0.7	1.0	0.9 ± 0.1
48	ND	ND	ND	ND	ND	
72	ND	ND	ND	ND	ND	
Application 2						
1 hour	—	—	—	—	—	
2	—	0.9	—	1.0	—	0.95
4	—	—	—	1.3	1.3	1.3
8	—	—	ND	ND	ND	
12	ND	ND	1.0	ND	—	
24	ND	ND	ND	1.0	ND	
48	ND	ND	ND	ND	0.8	

NF = None Found
ND = None Detected (limit of detection = 0.7 μg/L)
— = No sample
[a] = Concentrations are μg/L

recorded in the *in situ Diaptomus* bioassays after the second application.

Laboratory Toxicity Tests

The results of the laboratory static exposure are shown in Table 4. *Daphnia* was the most sensitive animal tested. A 24-hr LC_{50} could not be calculated from the laboratory data but a 24-hr exposure to 1.3 μg/L resulted in complete immobilization of test animals. To assess the effects of shorter exposures, daphnids were exposed for 4, 8, and 12 hr (Figure 8). With linear regression estimation of LC_{50}'s, values of 2.8, 1.3, and 0.4 μg/L are obtained for the 4, 8, and 12-hr exposure.

Diaptomus exposed for 20 hr at 6 μg/L showed an 85% mortality while a mixed cyclopoid copepod group exposed for 24 hr at just 2.9 μg/L showed the same level of mortality. The 5 hour LC_{50} for the mixed cyclopoid copepod group was 13.6 μg/L. Since 6 μg/L was the lowest concentration for the *Diaptomus* tests, a clear statement of differences with the cyclopoids is not possible. The copepod and *Chaoborus* data show the importance of exposure time in the assessment of effects. *Chaoborus* show an LC_{50} of 20.9 μg/L at 5 hr of exposure, but after 24 hr the LC_{50} is 1.2 μg/L and by 48 hr, 30% were immobilized or dead at 0.8 μg/L.

Discussion

Assessing the risk of a chemical to a natural aquatic system requires information in three distinct areas: first, data on the natural populations that may be exposed to typical use patterns; second, the laboratory toxicity of the agent; and third, an estimate of the chemical's concentration in the aquatic system. In this study, results from data gathered in these three areas show that the usual approach of measuring changes in population numbers in the natural habitat combined with data from 24 or 48 hr acute laboratory exposures may not provide critical information needed to assess the environmental risk of the agent for two reasons. First, longer term effects on reproduction and timing of resting stages are not usually detected in short-term measurements of changes in natural population densities. Second, agents that degrade rapidly within the first 24 hr of application require shorter term laboratory toxicity tests. In addition to population densities, it is important to measure reproduction, such as the absolute number of gravid females, clutch sizes, and the production of resting stages in relation to the typical timing of the applications of the pesticide, because populations appearing after an application may represent an investment of resting stages made by the organisms during the preceding season or year.

Table 3. Results of *in-situ* bioassays after temephos application I (22 May) and II (5 June, 1984) for *Daphnia pulex*, *Diaptomus* and *Chaoborus americanus*. % = dead or immobilized of 10 animals per test, n = number of assays. Hours (hr) are times post-application. Temephos concentrations given as µg/L average followed by range

	4 hr			24 hr			48 hr		
	%	n	µg/L	%	n	µg/L	%	n	µg/L
Application I									
Daphnia pulex	90	4	1.6 ± 0.7 (12)[d]	100[a]	5	1.2 ± 0.3 (15)[d]	100[b]	5	***
Diaptomus	0	2	2.6–0.7[e]	0	2	1.9–0.7[e]	0	1	
Chaoborus	0	1		90	3		4[c]	5	
Application II									
Daphnia pulex	60	5	1.2 ± 0.2 (4)[d]	100	5	1.1 ± 0.2 (5)[d]	100[b]	5	***
Diaptomus	0	3	1.3–0.9[e]	27	3	1.3–0.9[e]	53	3	
Chaoborus	0	5		13	3		26	3	
				80	2		45[c]	2	

[a] exposed from 6 to 24 hr
[b] exposed from 24 to 48 hr
[c] exposed from 29 to 48 hr
[d] $\bar{X}$ ± 1 Std Dev (n)
[e] Range of temephos concentrations
*** = Below detection limit of 0.7 µg/L

Although populations of *Daphnia pulex*, *Simocephalus*, Chydoridae and *Diaptomus leptopus* exhibited varying degrees of numerical recovery after treatment, analysis showed reproduction was strongly curtailed. The number of gravid females per m^3 compared with the previous year's reproduction was lower. For *Daphnia*, the number of gravid females during the post-application period was low in both ponds in 1984, but in 1983 there had been a strongly gravid population in the pretreatment pond. In 1984, in comparable periods, the treatment pond had no *Daphnia* while there was a definite population in the reference pond through June and the rest of the summer. An explanation for a decline in reproduction in June in the reference pond lies in the oxygen levels which were sharply reduced in May and June and reflected in the deep red hue of the *Daphnia* during this time, presumably from hemoglobin (Figure 9). Reproduction in terms of eggs/brood in *Daphnia* declines as O_2 goes below 2 ml/L (Fox *et al.* 1951). Oxygen depletion did not occur in the treated pond in May and June (Fig. 9), so temephos, not oxygen was the factor in the June loss of *Daphnia*.

Daphnia and *Simocephalus* that appeared 35 days post-treatment had hatched from diapausing eggs deposited prior to the temephos application. After the applications, gravid *Daphnia* appeared on only two dates in the treated pond. The reduction in females achieving reproductive condition, and the loss of a fall *Daphnia* population could result in a long-term population reduction. Some of the decline in *Daphnia* in September in the treated pond could have resulted from the comparatively lower oxygen levels from 20 August to 6 September.

Ephippial production of *Daphnia* occurred in 1983 in the fall, and in May of 1984 just before the application. If the application was earlier in May, the spring production of ephippia would not have occurred and the potential for recovery could have been depressed. Following the treatments, there was an unusual midsummer production of ephippia, so it appeared these *Daphnia* made ephippia as soon as they could reproduce. In Minnesota, ephippial populations occur most commonly in the fall, sometimes in the spring, but not in midsummer.

In 1983, gravid *Simocephalus* and Chydoridae were abundant in funnel trap samples (Figures 2C, 3B), whereas in 1984, post-application, none collected by funnel traps were gravid. In the plankton tows, reproducing individuals were collected in both 1983 and 1984. The absence of reproducing animals in the funnel traps in 1984 suggests that for some time after the applications, reproduction was inhibited in both chydorids and *Simocephalus* associated with the pond bottom.

Female *Diaptomus leptopus* at the application were preceded by a population of non egg-bearing adults. Development of a mature generation during March and April, before sampling began, could have occurred, because the generation times of 4.6 to 7.2 weeks have been reported for this species (O'Brien *et al.* 1973), and other diaptomids have

Table 4. Laboratory toxicity data from static exposure to temephos. Immobilized = lack of coordinated swimming ability or lying on the surface or bottom

	Hours Exposed	Concentration***	Effect	
			Immobilized	Dead
Cladocera				
Ceriodaphnia	4	5.2	100*	0
	24	5.2	0	100
Daphnia sp.	4	4.3	70	0
	5	4.1	60	40
	5	2.9	75	25
	24	2.9	0	100
	24	1.3	100	0
	24	0.8	0	10
	48	0.8	0	90
Copepoda				
Diaptomus sp.	20	6.0	0	85
Cyclopoida	5	4.1	0	5
	5	2.9	0	0
	5	—	LC_{50} = 13.6 (11.2–16.4)**	
	24	2.9	15	85
Diptera				
Chaoborus	5	—	LC_{50} = 20.9 (16.4–26.6)	
	12	—	LC_{50} = 2.4 (2.0–2.9)	
	24	—	LC_{50} = 1.2 (1.1–1.3)	
	24	0.8	0	5
	48	0.8	20	10
	72	0.8	40	30

* % affected
** LC_{50} in µg/L (95% Confidence Interval)
*** µg/L temephos

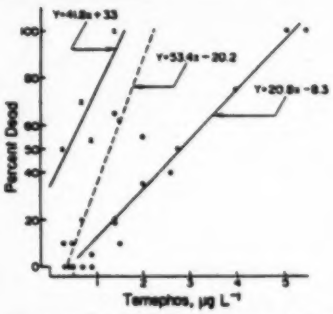

Fig. 8. Relationship of exposure time to survival of *D. pulex* in the laboratory for 4 (●), 8 (○) and 12 hours (x) of exposure

produced non-resting eggs under ice cover (Hairston and Olds 1984; Hairston 1987). The gravid adults first seen in June, therefore, came either from non-resting eggs produced by a mature generation earlier in the spring, or from the previous season's resting eggs.

The decrease in eggs per brood in *Diaptomus* in June 1984 compared with 1983 (28 vs 38) is probably not related to differences in temperature, and could be attributed to temephos. Water temperature was higher in spring 1983 than in spring 1984. Sawchyn and Hammer (1968) found the decrease in the number of eggs per female *D. leptopus* in Saskatchewan ponds was directly associated with an increase in water temperature. During June 1984, when water temperatures were lower than in June 1983, clutch size was reduced, and remained below those of June 1983, an effect opposite to that expected for *D. leptopus* in lower temperatures. This reduction in fecundity could be attributed to a change in food levels (not measured) or to the action of temephos. Smaller clutch sizes were observed later in the 1984 season when the temperature increased.

The June generation of *Diaptomus* is the major source of egg production. The sharp post-application reduction in the June-July egg production could result in fewer dispausing eggs to develop into subsequent populations. The exact effect of reduced June egg production is not clear, since differing patterns of diapause egg production occur for diaptomids and the eggs were not tested for dia-

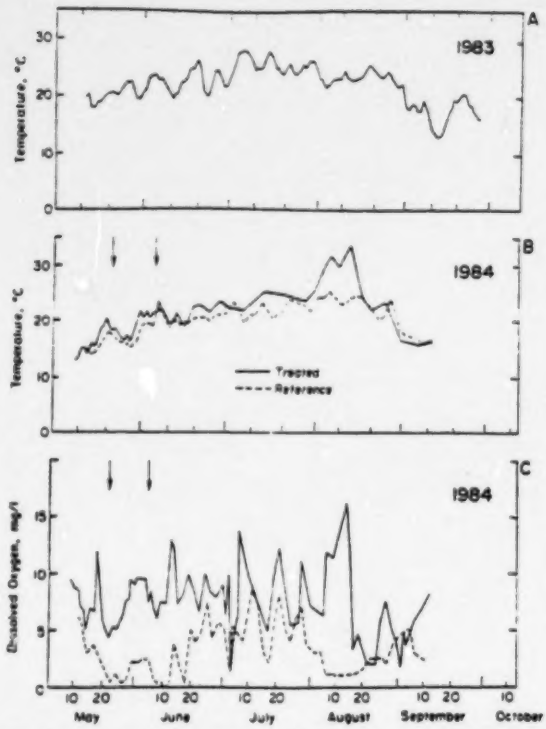

Fig. 9. Average daily water temperatures in 1983 and 1984 in treated (———) and reference (– – –) ponds, measured at 30 cm depth (A, B). Average daily dissolved O_2 in 1984 (C) at 30 cm

pause in this study. Generally, diapausing eggs are produced in summer in fishless ponds and in spring in ponds with fish (Hairston and Olds 1984; Hairston 1987).

Field Population Sensitivities to Temephos

Acute sensitivities of zooplankton were greatest for *Daphnia pulex*, *Simocephalus*, and Chydoridae followed by *Chaoborus*, and *Diaptomus*, all affected at 1–2 μg/L, the level used for mosquito control. Cyclopoid copepods, copepod nauplii and *Keratella cochlearis* were not sensitive at the concentrations achieved in the pond, with similar results on copepods seen by Ruber and Baskar (1968). The increase in *Keratella cochlearis* and copepod nauplii populations agrees with Papst and Boyer's (1980) results for temephos exposure, but could be attributed to normal population increases.

Daphnia pulex was the most sensitive animal tested in laboratory and *in situ* assays. The mortality, predicted from laboratory tests, from an 8 hr exposure is about 65% (Figure 8) while the field data at 8 hr show a decrease of 98% during an actual exposure of the *Daphnia* population exposed to >1.3 μg/L (Table 2). Concurrent with the decrease in *Daphnia* population, the *in situ* bioassays showed 90–100% mortality (Table 3), when concentrations were below the limit of detection (0.7 μg/L). The laboratory exposures show that 48 hr of exposure are necessary to produce 90% mortality at 0.8 μg/L. The *in situ* tests show an equal mortality to the laboratory tests but in less time and at lower concentrations. The loss of *Daphnia* from the pond for 35 days agrees with the result of Hughes *et al.* (1980). The additional mortalities in the pond cannot be explained by the laboratory data, but are suggestive of a higher sensitivity in the pond. A chronic effect concentration for *Daphnia* may be below the limit of analysis for temephos (0.7 μg/L). It is not known if temephos absorbs to particulate matter or is taken up by algae that would be ingested by the herbivorous filter-feeding zooplankton. But it is quite likely that the pond organisms initially experienced a wider range of concentrations of temephos as it mixed in the pond, since measurements of temephos showed a patchiness of distribution especially within the first hour after treatment.

Copepods show a varied response in the pond, *in situ* bioassays and laboratory tests. Pond populations of *Diaptomus* had immediate mortality, followed by depressed reproduction and apparent recovery of the adults. Ali and Mulla (1978) applied temephos at 9.2 μg/L and reported no effect, but their recovery population was only 23% of the control. While our pond populations of *Diaptomus* were reduced by 77% at 24 hr after the application, no *Diaptomus* died in the *in situ* bioassay chambers. *Diaptomus* laboratory tests were inconclusive, but the mixed cyclopoid copepod tests indicated 100% death or immobilization at 24 hr of exposure to 2.9 μg/L (Table 4). During the second pond exposure, the concentrations were lower, but the mortality of *Diaptomus* was higher in *in situ* assays (Table 3). The reason for this reversed result is not clear. As with *Daphnia*, *Diaptomus* showed greater sensitivity in the pond populations than in the laboratory assay.

The *Chaoborus* data show that these animals also have a greater sensitivity in the pond. The 24-hr LC_{50} is 1.2 μg/L, but 90% of the animals in the *in situ* bioassays were dead after a similar exposure in the pond. The *in situ* data is supported by an 84% decrease in the natural population. The second application shows that some containers had greater death rates; therefore, location may be important.

Other factors, such as temperature, may contribute to the difference, but if the warmer temperature increased the temephos toxicity, the effect was only seen with *Diaptomus*.

The high mortality of *Chaoborus* in pond populations, *in situ* assays and laboratory tests shows *Chaoborus* has a sensitivity to temephos similar to that of mosquito larvae. Its recovery may have been from new recruitment from eggs deposited by airborne adults. Whether the short-term reductions of this important predator of zooplankton had any long-term impact on this pond community is unknown.

In conclusion, the combined results of the three methods of measuring effects on *Daphnia*, *Diaptomus* and *Chaoborus* affirm the sensitivity of cladocerans and *Chaoborus* at concentrations used for insect control, and suggest the sensitivity of the plankton community to the pesticide may be greater in the pond than in the laboratory. The laboratory short-term (4 and 12 hr) tests were a conservative estimate of actual pond exposure results. The short-term laboratory tests display the important effect of exposure time, where lower concentrations have a greater effect when exposure times are longer. This dose-time response effect has been observed with temephos and other pesticides in both target (*Simulium*) and non-target species (Frommer *et al.* 1983; Anderson and Shubat 1984). This is why the degradation rate or persistence of the chemical in the field test environment needs to be assessed.

This study points to the importance of assessing reproduction and life cycle effects of non-target species. The usual approach of counting animal densities may not reveal the full impact of a pesticide in the aquatic community. In addition, development of short-term exposure techniques that reflect the actual environmental concentrations of the pesticide is needed. This kind of data is essential for analysis of effects in natural systems, especially for agents that have short-term residual times and are applied more than once a year.

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